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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/525,019	GIESING ET AL.	
	Examiner	Art Unit	
	Jennifer Dunston	1636	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *01 July 2009 and 24 July 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *26-68* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *26-68* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *18 February 2005* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 7/24/2009 has been entered.

Receipt is acknowledged of an amendment, filed 7/24/2009, in which claims 1-4, 6, 11, 12 and 14-25 were canceled, and claims 26-68 were newly added. Claims 26-68 are pending.

Election/Restrictions

Applicant elected Group I with traverse in the reply filed on 1/28/2008.

Claims 26-68 are under consideration.

Claim Objections

Claim 31 is objected to because of the following informalities: the phrase "any of claim 26" should be replaced with "claim 26" to improve the grammar of the claim. Appropriate correction is required.

Claim 66 is objected to because of the following informalities: (i) the word "and" should be placed between the first and second method steps at the end of line 23 of the claim; and (ii) at lines 29-30, the phrase "to indicate" should be replaced with "indicates". These changes will

improve the grammar of the claim. Claim 67 depends from claim 66 and is objected to for the same reasons applied to claim 66. Appropriate correction is required.

Claim 68 is objected to because of the following informalities: the term "TXNRD1" is misspelled at lines 15, 16 and 18 of the claim. Appropriate correction is required.

Response to Arguments - Claim Objections

The objections of claims 1, 21 and 23 are moot in view of Applicant's cancellation of the claims.

Double Patenting (Warning)

Applicant is advised that should claim 26 be found allowable, claim 31 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). Claim 26 is drawn to a "method for investigating a body fluid from a human subject having or suspected of having cancer for disseminated cancer cells" where the outcome of the method "indicates the presence of disseminated cancer cells in the body fluid." Claim 31 requires the method of claim 26 to be "for identifying disseminated cancer cells in the body fluid." Although the wording is slightly different, the indication or identification of the cancer cells in the body fluid is essentially the same thing.

Applicant is advised that should claim 34 be found allowable, claim 35 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). Claim 34 depends from claim 26, and recites, "wherein the elevated expression of at least one of said genes indicates a risk to develop a metastasis or a recurrence." Claim 35 depends from claim 34 and recites, "which is for estimating the risk to develop a metastasis or a recurrence." Although the wording is slightly different, the indication or estimation of risk of metastasis or recurrence seems to be the same thing.

Applicant is advised that should claim 50 be found allowable, claim 61 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). Claim 50 is drawn to a "method for investigating a body fluid from a human subject having or suspected of having cancer for disseminated cancer cells" where the outcome of the method "indicates the presence of disseminated cancer cells in the body fluid." Claim 61 requires the method of claim 50 to be "for identifying disseminated cancer cells in the body fluid." Although the wording is slightly different, the indication or identification of the cancer cells in the body fluid is essentially the same thing.

Applicant is advised that should claim 62 be found allowable, claim 33 will be objected to under 37 CFR 1.75 as being a substantial duplicate thereof. When two claims in an application are duplicates or else are so close in content that they both cover the same thing, despite a slight difference in wording, it is proper after allowing one claim to object to the other as being a substantial duplicate of the allowed claim. See MPEP § 706.03(k). Claim 62 depends from claim 50 and recites, "wherein the elevated expression of at least one of said genes indicates the presence of a tumor." Claim 33 depends from claim 50 and recites, "which is for diagnosis of a tumor." Claim 50 requires higher expression of at least one gene to indicate the presence of disseminated cancer cells in the body, and claim 33 requires this feature to diagnose a tumor. Claim 62 also requires elevated or higher expression of at least one gene to indicate the presence of a tumor. Thus, both claims cover the same thing.

Claim Rejections - 35 USC § 112

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claim 47-49 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 47 is vague and indefinite in that the metes and bounds of the phrase "if the ratio of its expression from the cell-containing fraction to the further cell-containing fraction is higher than the average ratio of its expression in subjects not having cancer" are unclear. A ratio is a proportional relationship between two different numbers or quantities. It is unclear what two

numbers or quantities make up the "average ratio of its expression in subjects not having cancer." Neither the specification nor claims define such an average ratio. The claim requires the gene expression to be part of the ratio, but the second part is undefined. Because the value of the ratio is relative to the second part, and the second part is not defined, the metes and bounds of the claim are unclear. Furthermore, Claim 47 depends from claim 26, which requires the indication of the presence of cancer cells when at least one of said at least 2 genes determined in the cell-containing fraction, as compared to its expression in the further cell-containing fraction, indicates the presence of disseminated cancer cells in the body fluid. Thus, two different standards are provided by claim 47 for indicating the presence of the cancer cells in the body fluid.

Claim 48 is vague and indefinite in that the metes and bounds of the phrase "if the ratio of its expression from the cell-containing fraction to the further cell-containing fraction is higher than the average ratio of its expression in subjects not having cancer" are unclear. A ratio is a proportional relationship between two different numbers or quantities. It is unclear what two numbers or quantities make up the "average ratio of its expression in subjects not having cancer." Neither the specification nor claims define such an average ratio. The claim requires the gene expression to be part of the ratio, but the second part is undefined. Because the value of the ratio is relative to the second part, and the second part is not defined, the metes and bounds of the claim are unclear.

Claim 49 depends from claim 48 and is thus rejected for the same reasons applied to claim 48.

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 26-68 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for (a) obtaining a blood sample from a human subject, collecting mononuclear cells from the blood sample, removing a fraction of the mononuclear cells to obtain test fraction A', passing the remaining mononuclear cells through a screen with a 20 μm mesh, and collecting cells from the mesh to obtain test fraction C;

(b) obtaining blood samples from a healthy human subjects not suffering from cancer, collecting mononuclear cells from the blood samples, removing a fraction of the mononuclear cells to obtain reference fraction A', passing the remaining mononuclear cells through a screen with a 20 μm mesh, and collecting cells from the mesh to obtain reference fraction C;

(c) isolating CD45-positive lymphocytes from reference fraction A' to obtain reference fraction A,

(d) isolating mRNA from test fraction A', test fraction C, reference fraction A, and reference fraction C;

(e) measuring the expression level of manganese superoxide dismutase (MNSOD), thioredoxin reductase (TXNRD1), and glutathione peroxidase (GPX1) in each of the mRNA samples, wherein said measuring is by reverse transcription and PCR using primers consisting of SEQ ID NOs: 1 and 2 for MNSOD, SEQ ID NOs: 4 and 5 for TXNRD1, and SEQ ID NOs: 7 and 8 for GPX1;

(f) determining the average and standard deviation for the expression ratio of MNSOD, TXNRD1, and GPX1 from reference fraction C to reference fraction A for the healthy control samples, and determining a limit for expression which is the average plus one standard deviation;

(i) determining the expression ratio of MNSOD, TXNRD1, and GPX1 from test fraction C to test fraction A' of the test sample; and

(j) comparing the expression ratio for each of MNSOD, TXNRD1, and GPX1 for the test sample to the determined limit for each gene;

wherein an expression ratio higher than the limit for at least one of MNSOD, TXNRD1 or GPX1 indicates that disseminated cancer cells are present in the test blood sample,

does not reasonably provide enablement for the use of bone marrow, the use of the method for a subject other than a human subject, the use of a further-cell containing fraction as a reference sample, the use of an undefined average ratio from subjects not having cancer, determining the expression of any manganese superoxide dismutase gene, any thioredoxin reductase gene, or any glutathione peroxidase gene, measuring protein levels to indicate the presence of disseminated cancer cells in the body fluid, diagnosis of a tumor, or estimating the risk to develop a metastasis or recurrence. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention commensurate in scope with these claims. This rejection was made in the Office action mailed 1/5/2009 and has been rewritten to address the amendments to the claims in the reply filed 7/24/2009.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the

existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

Nature of the invention: Claims 26-32, 34-47, 63 and 65 are drawn to a method for investigating a body fluid from a human subject having or suspected of having cancer for disseminated cancer cells. Claim 26 is drawn to the following method steps: (1) obtaining a cell-containing fraction from the body fluid with enrichment of cancer cells and determining in the cell-containing fraction from the expression of at least 2 genes which are selected from the group consisting of (i) a human manganese superoxide dismutase genes; (ii) a human thioredoxin reductase 1 genes; and (iii) a human glutathione peroxidase 1 genes, wherein the body fluid is blood or bone marrow; (2) providing a further cell-containing fraction of the body fluid from the same individual and determining the expression of the genes in the further cell-containing fraction; and (3) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its expression in the further cell-containing fraction, wherein an elevated expression of at least one of said at least 2 genes determined in the cell-containing fraction, as compared to the further cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid. The nature of the invention is complex in that increased expression of at least one of the claimed genes must be indicative of disseminated cancer cells in blood or bone marrow when any cell-containing fraction is compared to another cell-containing fraction from the same individual.

The dependent claims further limit the genes that are measured. Dependent claim 27 requires the expression of a manganese superoxide dismutase gene, a thioredoxin reductase gene

and a glutathione peroxidase 1 gene to be determined. Claim 30 requires the expression of a manganese superoxide dismutase gene and at least one further gene selected from a thioredoxin reductase 1 genes and a glutathione peroxidase 1 genes to be determined. Claim 36 requires the manganese superoxide dismutase gene to encode a protein that has the amino acid sequence of SEQ ID NO: 13 or an allelic variant thereof. Claim 37 requires the manganese superoxide dismutase gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2. Claim 38 requires the thioredoxin reductase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 15 or an allelic variant thereof. Claim 39 requires the thioredoxin reductase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 4 and SEQ ID NO: 5. Claim 40 requires the human glutathione peroxidase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 17 or an allelic variant thereof. Claim 41 requires the human glutathione peroxidase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8. Claim 42 specifically requires determining the expression levels by determining mRNA expressed by the genes.

The dependent claims further limit the step of obtaining a cell-containing fraction. Dependent claim 28 limits the body fluid to blood. Claim 29 limits the step of obtaining the cell-containing fraction to passing the body fluid or a cell-containing fraction thereof through a screen with a mesh or pore width of about 10 to 200 μm and obtaining the cell fraction retained on the screen. Claim 46 limits the method of claim 29 to the method where the screen has a mesh or pore width of about 20 μm. Claims 43 and 44 require the step of isolating cancer cells.

The nature of claims is complex in that they require the isolation of cancer cells from a subject only suspected of having cancer. Claim 45 limits the cell-containing fraction to one that is derived from blood and comprises mononuclear cells.

The dependent claims recite intended uses of the method. Claim 31 recites, "which is for identifying disseminated cancer cells in the body fluid." Claim 32 recites, "wherein the elevated expression of at least one of said genes indicates the presence of a tumor." Claim 63 depends from claim 44 and requires the method to be "for diagnosis of a tumor." The nature of claims 32 and 63 is complex in that it encompasses the initial diagnosis of tumors and diagnosis of a tumor that resulted from a metastasis or a recurrence (e.g., specification, pages 31-32). Claim 34 recites, "wherein the elevated expression of at least one of said genes indicates a risk to develop a metastasis or a recurrence. Claim 65 depends from claim 46 and recites, "which is for estimating the risk to develop a metastasis or recurrence. The nature of the invention is complex in that it seeks to further categorize individuals with disseminated cancer cells as having increased risk or not having increased risk to develop a metastasis or recurrence.

Claim 47 requires the elevates expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its expression in the further cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid if the ratio of its expression from the cell-containing fraction to the further cell-containing fraction is higher than the average ratio of its expression in subjects not having cancer. Claim 47 depends from claim 26, which requires the indication of the presence of cancer cells when at least one of said at least 2 genes determined in the cell-containing fraction, as compared to its expression in the further cell-containing fraction, indicates the presence of disseminated cancer cells in the body fluid.

Claims 48 and 49 are directed to a method for investigating a body fluid from a human subject having or being suspected of having cancer for disseminated cancer cells. The method of claim 48 comprises the following steps: (1) obtaining a cell-containing fraction from the body fluid by passing the body fluid or a cell-containing fraction thereof through a screen with a mesh or pore width of about 20 μm and obtaining the cell fraction retained on the screen, wherein the body fluid is blood or bone marrow; (2) determining in the cell-containing fraction the expression of (i) a human manganese superoxide dismutase gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2, wherein the manganese superoxide dismutase gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 13 or an allelic variant thereof; (ii) a human thioredoxin reductase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 4 and SEQ ID NO: 5, wherein the thioredoxin reductase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 15 or an allelic variant thereof; and (iii) a human glutathione peroxidase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8, wherein the human glutathione peroxidase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 17 or an allelic variant thereof; (3) providing a further cell-containing fraction of the body fluid from the same individual and determining the expression of the genes in the further cell-containing fraction of the body fluid; and (4) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its expression in the further cell-containing fraction, wherein an elevated expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its expression in

the further cell-containing fraction indicates the presence of disseminated cancer cells in the body fluid if the ratio of its expression from the cell-containing fraction to the further cell-containing fraction is higher than the average ratio of its expression in subjects not having cancer. Claim 49 depends from claim 48 and requires the following: (a) the body fluid is blood; (b) the cell-containing comprises mononuclear cells; (c) the further cell-containing fraction comprises mononuclear cells; and (d) determining the expression comprises determining mRNA expressed by the gene. The nature of the invention is complex in that a partially undefined comparison is used in making the determination of whether disseminated cancer cells are present in the body fluid.

Claims 50-62, 64 and 33 are drawn to a method for investigating a body fluid from a human subject having or being suspected of having cancer for disseminated cancer cells. Claim 50 sets forth the following method steps: (1) obtaining a cell-containing fraction from the body fluid and determining in the cell-containing fraction the expression of at least 2 genes which are selected from the group consisting of (i) a human manganese superoxide dismutase gene; (ii) a human thioredoxin reductase 1 gene; and (iii) a human glutathione peroxidase 1 gene, where the body fluid is blood or bone marrow; and (2) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its average expression in subjects not having cancer, wherein higher expression of at least one of said at least 2 gene sin the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body fluid.

The dependent claims further limit the genes that are measured. Claim 51 requires the expression of a manganese superoxide dismutase gene and at least one further gene selected from

a thioredoxin reductase 1 genes and a glutathione peroxidase 1 genes to be determined. Claim 52 requires the expression of a manganese superoxide dismutase gene, a thioredoxin reductase gene and a glutathione peroxidase 1 gene to be determined. Claim 55 requires the manganese superoxide dismutase gene to encode a protein that has the amino acid sequence of SEQ ID NO: 13 or an allelic variant thereof. Claim 56 requires the manganese superoxide dismutase gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 1 and SEQ ID NO: 2. Claim 57 requires the thioredoxin reductase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 15 or an allelic variant thereof. Claim 58 requires the thioredoxin reductase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences set forth in SEQ ID NO: 4 and SEQ ID NO: 5. Claim 59 requires the human glutathione peroxidase 1 gene to encode a protein having the amino acid sequence of SEQ ID NO: 17 or an allelic variant thereof. Claim 60 requires the human glutathione peroxidase 1 gene to encode an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8. Claim 54 specifically requires determining the expression levels by determining mRNA expressed by the genes.

The dependent claims recite intended uses of the method. Claim 61 recites, "which is for identifying disseminated cancer cells in the body fluid." Claim 62 recites, "wherein the elevated expression of at least one of said genes indicates the presence of a tumor." Claim 33 depends requires the method to be "for diagnosis of a tumor." The nature of claims 62 and 33 is complex in that it encompasses the initial diagnosis of tumors and diagnosis of a tumor that resulted from a metastasis or a recurrence (e.g., specification, pages 31-32). Claim 64 recites, "wherein the elevated expression of at least one of said genes indicates a risk to develop a metastasis or a

recurrence. The nature of the invention is complex in that it seeks to further categorize individuals with disseminated cancer cells as having increased risk or not having increased risk to develop a metastasis or recurrence.

Claims 66 and 67 are drawn to a method for investigating a body fluid for disseminated cancer cells in a subject having or being suspected of having cancer. Claim 66 sets forth the following method steps: (1) obtaining a cell-containing fraction from the body fluid and determining in the cell-containing fraction the expression of (i) a human manganese superoxide dismutase gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 1 and SEQ ID NO: 2, wherein the manganese superoxide dismutase gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 13 or an allelic variant thereof; (ii) a human thioredoxin reductase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 4 and SEQ ID NO: 5, wherein the thioredoxin reductase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 15 or an allelic variant thereof; and (iii) a human glutathione peroxidase 1 gene which encodes an mRNA which is capable of being amplified using the primer sequences as set forth in SEQ ID NO: 7 and SEQ ID NO: 8, wherein the human glutathione peroxidase 1 gene encodes a protein having an amino acid sequence as set forth in SEQ ID NO: 17 or an allelic variant thereof, wherein the body fluid is blood or bone marrow; and (2) comparing the expression for each of said at least 2 genes in the cell-containing fraction with its average expression in subjects not having cancer, wherein higher expression of at least one of said at least 2 genes in the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body

fluid. Claim 67 depends from claim 66 and requires (i) the body fluid is blood; (ii) the cell-containing fraction comprises mononuclear cells; and (iii) determining gene expression comprises determining mRNA expressed by the gene.

Claim 68 is drawn to a method for investigating a blood or bone marrow sample for disseminated cancer cells in a human subject having or suspected of having cancer. The claim sets forth the following method steps: (a) obtaining a blood or bone marrow sample from the human to obtain a test fraction; (b) obtaining a blood or bone marrow sample from a healthy human subject not suffering from cancer to obtain a reference fraction; (c) isolating mRNA from the test fraction and reference fraction to obtain an mRNA test sample and an mRNA reference sample, respectively; (d) measuring the expression level of human manganese superoxide dismutase (MNSOD), human thioredoxin reductase 1 (TXNRD1), and human glutathione peroxidase 1 (GPX1) in the mRNA sample and the mRNA reference sample, wherein the measuring is by reverse transcription and PCR with primers selected from the nucleotides of SEQ ID NOs: 1 and 2 for MNSOD; SEQ ID NOs: 3 and 4 for TXNRD1; and SEQ ID NOs: 7 and 8 for GPX1; and (e) comparing the expression of MNSOD, TXNRD1, and GPX1 in the mRNA test sample to the mRNA reference sample, and wherein a higher expression of MNSOD, TXNRD1 and GPX1 in the **mRNA test sample** as compared to the **mRNA test sample** indicates the presence of disseminated cancer cells in the blood or bone marrow samples from the human subject having or suspected of having cancer. The nature of the invention is complex in that comparing the test sample to the test sample should result in the same level of expression and not higher or lower expression.

The nature of the invention is complex in that carrying out the recited method steps must enable the intended uses of the method, including identifying disseminated cancer cells in a body fluid, providing a diagnosis of a tumor, and estimating the risk to develop a metastasis or recurrence.

Breadth of the claims: The claims are broad in that the specification defines the term "cancer cell" to mean a cell which exhibits one or more modifications associated with cancer, i.e., dysplasia in the general sense. The term is defined to specifically include precursors of cancer and tumor cells with cancerous or tumorous modifications (e.g., page 4, lines 6-19).

The claims are very broad in that they encompass determining the expression of at least two genes selected from manganese superoxide dismutase genes, thioredoxin reductase 1 genes, and glutathione peroxidase 1 genes. The specification defines the term "manganese superoxide dismutase (MNSOD)" to mean enzymes which catalyze the decomposition of superoxide free radicals to form hydrogen peroxide, and in particular the enzymes which constitute enzyme class 1.15.1.1 (paragraph bridging pages 14-15). The enzymes of this class are not limited to manganese-containing superoxide dismutase enzymes (See the entry for 1.15.1.1 from the Enzyme nomenclature databases, accessed from http://us.expasy.org/enzyme, cited in a prior action). Enzymes of the class 1.15.11 include all superoxide dismutase enzymes, including iron or manganese or copper and zinc superoxide dismutase. Thus, the claims read on determining the expression level of any superoxide dismutase enzyme from any species of organism from which a body fluid may be obtained. The claims read on determining the expression level of any thioredoxin reductase 1 isoform from any species of organism from which blood or bone marrow may be obtained. The claims read on determining the expression level of any glutathione

peroxidase isoform from any species of organism from which blood or bone marrow may be obtained. Accordingly, the claims broadly encompass obtaining blood or bone marrow from any species of organism, and determining the expression of at least two of the broadly defined classes of genes selected from the genus of manganese superoxide dismutase genes, the genus of thioredoxin reductase 1 genes, and the genus of glutathione peroxidase 1 genes. Claims 66 and 67 do not require the use of the primers in the claimed method. The gene must only be capable of being amplified by the primer sets. As shown in Exhibits I and II (mailed 1/5/2009), the primers of SEQ ID NOS: 7 and 8 are capable of amplifying genes from a number of different species.

The claims are broad in that the comparable biological sample may be from any body fluid or solid tissue of any subject. Accordingly, the claims encompass a large number of different comparisons between the tested cell-containing fraction and a further cell-containing fraction or a comparable biological sample. The claims also are drawn to the use of an "average ratio of its expression in subjects not having cancer" where the claims do not define the second value used to calculate the ratio.

The complex nature of the subject matter of this invention is greatly exacerbated by the breadth of the claims.

Guidance of the specification and existence of working examples: The specification envisions using a method for investigating body fluids for cancer cells to permit reliable tumor diagnosis and prognosis (e.g., page 1, lines 5-13).

The specification teaches that the prior art shows that some solid tumors and metastases thereof found in solid tissue have increased expression of MNSOD, including colorectal tumors

and hepatic metastases thereof, lung tumors, breast cancer cells, stomach tumors, and gliobalastoma (e.g., page 2, lines 5-22). However, the specification also notes that benign hyperplasias of the breast were often found to be strongly positive for MNSOD expression as compared to neoplastic epithelial cells from invasive carcinomas of the breast (e.g., page 2, lines 22-27). Thus, the specification acknowledges that MNSOD levels are not always higher in dysplastic cells as compared to any cell type. The specification teaches that reduced GPX1 expression was observed in imexon-resistant RPM/8226/I myeloma cells (e.g., page 3, lines 10-12). Thus, gene expression may vary depending upon the sensitivity or resistance of the cancer cell to a cancer therapeutic. Further, the specification teaches that disseminated cancer cells are a tumor entity independent of the primary tumor and therefore are fundamentally different from cells of the primary tumor on the basis of a different genotype and phenotype (e.g., page 3, lines 14-22).

At pages 12-27, the specification provides general guidance directed to measuring expression levels of MNSOD, TXNRD and GPX expression by measuring nucleic acid or protein expression.

At pages 28-30, the specification provides guidance with regard to evaluating the obtained expression levels. The specification teaches that it is particularly important to determine whether expression in the cells of the investigated sample is comparatively elevated (e.g., page 28, lines 1-10). The specification teaches that the comparison usually is with cells in which no cancer-associated modification is to be expected (non-cancer cells, normal cells) (e.g., page 28, lines 10-14). The specification suggests that if cancer cells in body fluids are being tested, then the comparison will be those normally occurring in this body fluid. For the case of

blood, the normal cells are white blood cells which can be obtained for example by density gradient centrifugation (e.g., the buffy coat or the MNC fraction) or can be separated by more specific isolation methods (e.g., CD45-positive lymphocytes) (e.g., page 28, lines 14-22). The specification asserts that these samples can also be used as a comparison for body fluids other than blood (e.g., page 28, lines 22-25). At page 29, lines 33-39, the specification states, "The test principle according to the invention is therefore based on determining whether enrichment of cancer cells is associated with a measurable increase in MNSOD, TXNRD and GPX expression. The ratio of the expression measured in the test cell mixture to the expression measured in the comparison cell mixture is decisive." The specification goes on to state, "It will usually be expedient for validation of a particular test system to fix a particular quotient (limit) above which overexpression is present by definition." (See page 30, lines 1-5). Thus, the step of comparing appears to be critical to the claimed invention. Furthermore, the specification notes that the limit may depend on the cell mixtures used and, in particular, on the obtaining thereof (e.g., page 30, lines 7-8).

With respect to early diagnosis, the specification envisions using sputum/saliva for the early diagnosis of lung tumors; urine for the early diagnosis of prostate and bladder tumors; stool for the early diagnosis of colonic and pancreatic tumors; and blood/bone marrow/lymph for the early diagnosis of all disseminating tumors.

With respect to the prognosis and risk of recurrence, the specification envisions using the method of the invention to classify tumor and estimate risk (e.g., paragraph bridging pages 31-32).

The working examples of the specification are directed to one embodiment that falls within the scope of the instant claims. The examples teach the collection of blood from 9 healthy donors and 47 tumor patients. Breast carcinoma cell line BT474 was used as a reference for MNSOD, TXNRD1, and GPX1 expression. To obtain cancer cell fraction C and comparative fractions A' and B', 10 ml of heparinized blood was centrifuges, and the supernatant plasma was removed. The pelleted cells were resuspended in 12 ml of PBS and subjected to density gradient centrifugation. The mononuclear cell fraction was collected, washed and resuspended in 10 ml of PBS. 1 ml of this cell mixture was removed as a possible reference (comparative fraction A'). The remaining 9 ml of cell mixture was passed via a column through a screen woven from polyester filaments with a 20 μm mesh width, and the flow-through from the screen was collected as a possible reference (cell fraction B'). The column was washed five times with 10 ml of PBS, and the cells trapped on the screen were collected in Trizol® solution (cancer cell fraction C). Comparative fractions A' and B' were further processed by isolating CD45-positive lymphocytes to obtain comparative fractions A and B. Gene expression was analyzed by TaqMan® analysis of mRNA expression using the following primers and probes: SEQ ID NO: 1 (sense primer for MNSOD), SEQ ID NO: 2 (antisense primer for MNSOD), SEQ ID NO: 3 (probe for MNSOD), SEQ ID NO: 4 (sense primer for TXNRD1), SEQ ID NO: 5 (antisense primer for TXNRD1), SEQ ID NO: 6 (probe for TXNRD1), SEQ ID NO: 7 (sense primer for GPX1), SEQ ID NO: 8 (antisense primer for GPX1), SEQ ID NO: 9 (probe for GPX1), SEQ ID NO: 10 (sense probe for GAPDH), SEQ ID NO: 11 (antisense probe for GAPDH), and SEQ ID NO: 12 (probe for GAPDH). The specification refers to the following accession numbers for MNSOD, TXNRD1, and GPX1: M36693, X91247, and M21304, respectively. GAPDH

expression was measured for fractions A or A' and C, and the ratio of the expression of each gene is expressed as a quotient. The specification teaches that overexpression of the relevant mRNA is present if the ratio of the fraction C quotient to the fraction A quotient is more than a limit which is to be experimentally defined. Further, the specification teaches that cell equivalents are based on a cell standard (e.g., cell line BT474), where cDNA from the cell standard is included in the quantitative analysis in the form of serial dilutions and serves as a reference system (e.g., page 44, line 31 to page 45, line 1). The specification teaches the amounts of MNSOD, TXNRD1 and GPX1 mRNA determined in fraction C as compared to fraction A for healthy donors (e.g., Table 1). The specification teaches that for subsequent assessment of the levels of expression measured in tumor patients, levels were regarded as positive if they exceeded the average level in healthy donors (ratio of level in fraction C as compared to fraction A) plus one standard deviation, as indicated as the limit in Table 1 (e.g., page 45, lines 13-26). MNSOD, TXNRD1 and GPX1 was measured in fractions C and A' obtained from the blood of patients diagnosed with a solid tumor (e.g., page 46, lines 1-8). Comparing the expression ratios from fractions C and A' to the limits disclosed in Table 1, it was determined that 78/90 (87%) patients were positive for increased MNSOD expression, 60/90 (67%) of patients were positive for increased TXNRD1 expression, and 53/86 (62%) of patients were positive for GPX1. At least one gene was positive in 93% of patients. Thus, detecting all three genes has a sensitivity of 93%, while the sensitivity of the individual detections is 87, 67 and 62%, respectively (e.g., page 47, lines 25-30). Comparison between the healthy donors and some of the tumor patients is shown at pages 50-51. The specification teaches the use of this specific method to detect disseminated cancer cells in patients with solid tumors.

The specification does not teach the stage or grade of the cancers at the time blood was drawn. There is no indication that the cancer cells detected by increased expression of MNSOD, TXNRD1 or GPX1 are not a result of advanced metastatic cancer. The specification does not teach the sensitivity of the assay for early, non-metastatic cancer.

With respect to estimating the risk to develop metastasis or recurrence, the specification teaches the comparison between tumor patients with out recurrence and those with recurrence in relation to MNSOD, TXNRD1, and GPX1 expression as discussed above (e.g., pages 52-53). While some statistical differences were observed, the percentages disclosed in Table 7a for carcinoma of the breast and Table 7b for tumor patients, indicates that may not be able to use the expression levels of MNSOD, TXNRD1 and/or GPX1 to reliably classify a single test individual as at risk or not at risk of recurrence. The statistics presented are for groups of patients. All patient groups tested have levels higher than that of the normal controls (see tables). Thus, the indication of a tumor, a recurrence or progression is not based upon the presence or absence of disseminated cancer cells in the blood. Rather, the specification seeks to further classify those individuals with disseminated cancer cells.

The specification discloses probes that could be used for microarray analysis of MNSOD, GPX2, GPX3, and TXNRD1 (e.g., page 55). The specification asserts that overexpression of MNSOD and GPX2 is clearly evident upon hybridization of mRNA total amplification from a tumor cell fraction C as compared to cell fraction A' (e.g., page 57 and Figure 1).

The specification does not teach the expression of MNSOD, TXNRD or GPX in body fluids such as bone marrow, lymph, sputum, lavages, puncture fluids, ascites, mucosal smears,

exudates, urine or stool. The specification does not contain working examples directed to the diagnosis of a tumor or risk of developing a metastasis in a single human test subject.

Predictability and state of the art: The art teaches that gene expression analysis is commonly used for three different purposes: (1) as a screening tool to identify individual genes of interest that might contribute to an important biological function, (2) to obtain insight into an important biological function, and (3) as a classification tool to sort cases into clinically important categories (Pusztai and Hess, Annals of Oncology, Vol. 15, pages 1731-1737, 2004, cited in a prior action; e.g., paragraph bridging pages 1732-1733). Pusztai and Hess teach that validation of gene expression important to biological function may be validated by using different methods, such as RT-PCR, whereas the most appropriate validation for using gene expression analysis as a classification tool is testing the predictor on independent sets of cases (e.g., page 1733, left column, 1st full paragraph). In the instant case the specification does not teach that the expression levels can be used to reliably categorize an individual (other than indicates in the allowable scope). For example, the specification does not teach the classification of individuals as at risk or not at risk to develop a metastasis, or at risk to develop a recurrence.

Further, Shalon et al (US 2001/0051344 A1, Dec 13, 2001, cited in a prior action) teach that due to variations in genetic make-up of unrelated individuals in a heterogeneous society, differences in the expression of a gene between any two individuals may or may not be significant (e.g., paragraph [0155]). Shalon et al further teach that the larger the number of individuals tested, the more significant the remaining differences in gene expression become and samples from at least 5 and preferably 20-50 different test individuals are assayed to obtain statistically meaningful data showing a statistical elevation or reduction in report levels when

compared to control levels (e.g., paragraph [0156]). Pusztai and Hess teach that larger samples sizes may be needed to validate classification tests, and the number of samples will vary depending upon the acceptable error rates, level of inter-patient variability, the size of the difference in mean expression values, and the prevalence of the phenotype among the group being tested (e.g., page 1734, paragraph bridging columns; Table 1).

Genetic tests are heterogeneous in nature and the exact characteristics of a particular genetic test to be evaluated must be tightly defined (Kroese et al (Genetics in Medicine, Vol. 6, pages. 475-480, 2004, cited in a prior action). Kroese et al teach that genetic test is shorthand to describe a test to detect a particular genetic variant for a particular disease in a particular population and for a particular purpose and that it should not be assumed that once the characteristics of a genetic test are evaluated for one of these reasons that the evaluation will hold or be useful for other purposes and all measures of the test performance should be presented with their 95% confidence intervals (e.g., page 477, 1st column, 1st and 2nd full paragraph). Kroese et al teach that the limitations of our genetic knowledge and technical abilities means that for the moment there are likely to be gaps in the information needed to complete a thorough evaluation of many genetic tests (e.g., page 479, 2nd column, last paragraph).

The prior art reveals that differences in gene expression observed between two groups are do not necessarily provide markers that can be used to reliably classify a subject. Golub et al (Science, Vol. 286, pages 531-537, October 1999, cited in a prior action) teach the use of a two-step procedure to test the validity of gene expression levels as predictors: step 1 involves cross-validation of the predictors on the initial data set, where one withholds a samples, builds a predictor based only on the remaining samples and predicts the class of the withheld sample; step

2 involves the repetition of assessing the clinical accuracy of the predictor set on an independent set of samples (e.g., page 532, right column). Although Golub et al could detect gene expression differences between chemotherapy responders and non-responders, those differences could not be use to predictably classify individuals (e.g., page 533, paragraph bridging left and middle columns). Accordingly, the art demonstrates the unpredictable nature of extrapolating gene expression differences to a method of class prediction.

The art teaches that different isoforms of MNSOD, TXNRD1 and GPX1 are expressed (See the Entrez Gene entries for SOD2, TXNRD1, and GPX1 downloaded from http://www.ncbi.nlm.nih.gov/sites/entrez?db=gene on 5/5/2008, cited in a prior action). These genes correspond to the genes detected by the primers recited in the present specification. The specification does not specifically teach the increased expression of each isoform in circulating cancer cells. Thus, it would be unpredictable to detect any isoform of these genes for the use of the claimed method. Moreover, Seven et al (Clinical Biochemistry, Vol. 32, No. 5, pages 369-373, 1999, cited in a prior action) teach that the constitutive levels and the inducibility of antioxidant enzymes including superoxide dismutase and glutathione peroxidase vary for different tissues, and the expression of these enzymes may vary according to the type of cancer or tissue studies, resulting in controversy in the literature (e.g., page 372, left column, last two paragraphs). Seven et al did not find an increased amount of CuZn SOD or glutathione peroxidase in the red blood cell fraction of laryngeal cancer patients (e.g., page 372, paragraph bridging columns; Table 1). Furthermore, the post filing art teaches that the expression of MNSOD, TXNRD1 and GPX1 measured by medium density micorarray and real-time RT-PCR showed a poor correlation (Giesing et al. BJU International, DOI: 10.1111/j.1464-

410X.2009.08920.x , published online 10/10/2009, as pages 1-11; e.g., page 2, middle column). The microarray did not generate the necessary sensitivity and specificity to detect circulating cancer cells (e.g., page 3, right column, last full paragraph).

Amount of experimentation necessary: The quantity of experimentation necessary to carry out the full scope of the invention is large. One would be required to conduct a large number of experiments to test the expression of many manganese superoxide dismutase genes, thioredoxin reductase 1 genes, and glutathione peroxidase 1 genes, in combinations of two, from cell-containing fractions of body fluid, where the body fluid is blood or bone marrow, in a number of different species of organisms. Given the variable expression of the enzymes based upon tumor or cell type and the expression of multiple different isoforms, it would be unpredictable to extrapolate the results of the present specification to the use of any manganese superoxide dismutase gene, any thioredoxin reductase 1 gene, and/or any glutathione peroxidase 1 gene, and any comparative tissue. As discussed in the present specification, the limit used to determine whether a gene is overexpressed must be experimentally determined for each particular comparison. This comparison will be specific for the organism, body fluid, cells collected from the body fluid, gene whose expression is determined, isoform whose expression is determined, whether mRNA or protein expression is measured, the specific method used to measure the mRNA or protein (e.g., RT-PCR, microarray, or ELISA), whether enrichment is used, and the type of control sample. A large amount of unpredictable experimentation would be required to use the full scope of the claimed method to detect the presence of disseminated cancer cells, provide diagnosis of a tumor, estimate the risk to develop a metastasis, or estimate the risk to develop a recurrence.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 26-68 are not considered to be fully enabled by the instant specification.

Response to Arguments - 35 USC § 112

The rejection of claims 23 and 24 under 35 U.S.C. 112, second paragraph, is moot in view of Applicant's cancellation of the claims in the reply filed 7/24/2009.

The rejection of claims 1-4, 6, 11, 12 and 14-25 under 35 U.S.C. 112, first paragraph, is moot in view of Applicant's cancellation of the claims.

With respect to the rejection of claims 26-68 under 35 U.S.C. 112, first paragraph, Applicant's arguments filed 7/1/2009 have been fully considered but they are not persuasive.

At the last paragraph of page 13, the response indicates that claims 26-68 satisfy the enablement requirement for the reasons set forth in the present amendment and the amendment of April 13, 2009.

The arguments presented in the amendment filed April 13, 2009 were addressed in the Office action mailed 4/24/2009.

At page 14, the response indicates that claims 50-67 have been added, because comparing the expression for each of the at least two genes in the cell-containing fraction with its expression in the further cell-containing fraction a comparison is made with the subjects own non-cancer cells (page 29, lines 14-31) is asserted to be advantageous, but not mandatory. Further, the response notes that according to claims 50-67, a higher expression of at least one of the genes in

the cell-containing fraction as compared to its average expression in subjects not having cancer indicates the presence of disseminated cancer cells in the body fluid. The response asserts that this is sufficient. Moreover, the response notes that to make such comparisons, one skilled in the art has to know the average expression of each of the genes in the healthy subjects. The response indicates that this requires determining the expression in a number of healthy subjects (e.g., Example 1 of the specification). However, the response asserts that once the average expression in healthy subjects is known, the claimed method can be carried out without the need of repeating the determination. This is the rationale provided for not claiming this particular method step.

These arguments are not found persuasive. As indicated in the prior actions, the enabled scope of the claimed invention includes a comparison of gene expression levels of the claimed genes in a blood sample of a test human subject to the gene expression levels of the claimed genes in healthy subjects not having cancer. The working examples of the specification teach the calculation of the average and standard deviation of the expression level for each of the genes (e.g., Example 1). The specification states, "For the subsequent assessment of the levels of expression measured in tumor patients, the levels regarded as positive are those which exceed the average level plus standard deviation." The specific levels are disclosed in Table 1 at page 45 of the specification. These levels can be used without being calculated again, but only if the exact same conditions are used to calculate the expression levels. For instance, the specification teaches that for the evaluation of gene expression, the ratio of cell equivalents of the mRNA to be determined to cell equivalents of GAPDH is found for each cellular fraction, where the cell equivalents are based on a cell standard (e.g., page 44, line 23 to page 45, line 1). To obtain the

specific limits for MNSOD, TXNRD1, and GPX1 disclosed in Table 1 (i.e., 1.2, 1.3 and 5.2, respectively), mRNA extracted from a known number of cells of a cell suspension of cell line BT474 was used. The cDNA from the cell line was included in the form of serial dilutions to serve as a reference system (e.g., page 44, line 31 to page 45, line 1). If different numbers of cells are used as a reference or a different cell line is used as a reference, for example, the determined expression levels cannot be compared to the disclosed limits. One must calculate the average and standard deviation for the specific conditions used. The calculation of the limit is essential in obtaining a reliable classification of subjects as having or not having disseminated cancer cells in blood (e.g., Example 2). Claims 50-67 encompass the use of any tissue type from the subjects not having cancer as the comparison and are drawn to a comparison with the average expression level, rather than the average plus one standard deviation. Expression of the genes will be variable based upon the cell type selected. Thus, higher expression in the sample from the test subject will not be a reliable indicator of the presence of disseminated cancer cells. The specification does teach that it is particularly important to determine whether expression in the cells of the investigated sample is comparatively elevated (e.g., page 28, lines 1-10). The step of comparing is critical to the outcome of the invention, and it must be determined experimentally whether any particular comparison will provide a reliable indicator of the presence of a tumor or the risk of metastasis or recurrence. The Examiner has set forth evidence of the unpredictability of the invention above and in prior actions. Given the breadth of the claims, and the unpredictability in extrapolating the results disclosed in the working example to the full scope of the claimed invention, it would require a large amount of unpredictable experimentation to practice the full scope of the claimed invention.

At pages 14-19, the response compares the scope of the present claims with the scope considered to be enabled by the Examiner as set for the in the scope of enablement rejection made in the Office action mailed 1/5/2009. Each of the pending claims is broader in some aspects as compared to the enabled scope. The response asserts that the claimed method is enabled for any cell-containing fraction from blood or bone marrow; measuring mRNA by any method or measuring protein, because mRNA is translated into protein; and comparison with a further cell-containing fraction isn't necessary.

These arguments are not found persuasive. As discussed above, the comparison made is essential to the successful practice of the invention. The specification does not enable each of the claimed comparisons for the reasons set forth above and the reasons of record. Furthermore, the post filing art indicates that it would be unpredictable to extrapolate the RT-PCR results disclosed in the present specification to other methods of expression detection. Specifically, the post filing art teaches that the expression of MNSOD, TXNRD1 and GPX1 measured by medium density micorarray and real-time RT-PCR showed a poor correlation (Giesing et al. BJU International, DOI: 10.1111/j.1464-410X.2009.08920.x , published online 10/10/2009, as pages 1-11; e.g., page 2, middle column). The microarray did not generate the necessary sensitivity and specificity to detect circulating cancer cells (e.g., page 3, right column, last full paragraph).

At page 15, the response notes that claims 47 and 48 do not comprise step (b), because the average has to be determined only once. However, the claims do not contain a step of determining the average even once. The specification only sets for the limits for very specific conditions which are much narrower in scope than the present claims. Thus, one could not use the disclosed limits as the limits the claimed methods.

At page 19, the response asserts that Seven et al do not provide evidence of the unpredictable nature of the invention, because the claimed method is directed to the detection of disseminated cancer cells, not to assessment of antioxidant markers in plasma, erythrocytes or tumor tissue. Further, the response notes that although disseminated cancer cells are usually derived from a solid tumor, they are regarded as an independent tumor entity once circulating in the body fluid of an individual.

These arguments are not found persuasive. The claims are drawn to or encompass the detection of expression of mRNA or protein of (i) a human manganese superoxide dismutase gene, (ii) a human thioredoxin reductase 1 gene, and (iii) a human glutathione peroxidase 1 gene in blood or bone marrow. Plasma and erythrocytes are components of blood. Erythrocytes may also be found in bone marrow. Although the working example of the specification teaches the use of mononuclear cells isolated from blood, the claims are not limited to the use of these cells.

At the 1st paragraph of page 20, the response notes that the working examples show that irrespective of the tumor tissue from which the circulating tumor cells are derived, the disseminated cancer cells over-expressed the genes at stake.

The Examiner has indicated that the enabled scope is directed to any tumor type. The Examiner has not limited the enabled scope to any particular type of tumor.

At the second paragraph of page 20, the response asserts that even if it were assumed that the level of antioxidants may depend on the tissue studies, the present specification provides evidence that cancer cells in general are characterized by an elevated expression of at least one of the genes at stake.

This argument is not found persuasive. The increased expression is relative to the specific comparison made in the working examples. The evidence on the record does not demonstrate that selection of a different cell type for comparison, with a different level of gene expression for the genes at stake will result in the same level of sensitivity and specificity for the test. At page 29, lines 33-39, the specification states, "The test principle according to the invention is therefore based on determining whether enrichment of cancer cells is associated with a measurable increase in MNSOD, TXNRD and GPX expression. The ratio of the expression measured in the test cell mixture to the expression measured in the comparison cell mixture is decisive." The specification goes on to state, "It will usually be expedient for validation of a particular test system to fix a particular quotient (limit) above which overexpression is present by definition." (See page 30, lines 1-5). Thus, the step of comparing appears to be critical to the claimed invention. Furthermore, the specification notes that the limit may depend on the cell mixtures used and, in particular, on the obtaining thereof (e.g., page 30, lines 7-8). This is consistent with the teachings of the post filing art. Specifically, the post filing art teaches that the expression of MNSOD, TXNRD1 and GPX1 measured by medium density micorarray and real-time RT-PCR showed a poor correlation (Giesing et al. BJU International, DOI: 10.1111/j.1464-410X.2009.08920.x , published online 10/10/2009, as pages 1-11; e.g., page 2, middle column). The microarray did not generate the necessary sensitivity and specificity to detect circulating cancer cells (e.g., page 3, right column, last full paragraph). Furthermore, the claims encompass the use of MNSOD genes other than SOD2. Applicant has acted as his own lexicographer to specifically define the term "manganese superoxide dismutase (MNSOD)" to encompass both MNSOD and CuZnSOD even though this would be contrary to the ordinary

meaning of manganese superoxide dismutase (MNSOD). The written description clearly redefines the claim term and sets forth the uncommon definition so as to put one reasonably skilled in the art on notice that the applicant has intended to so define the claim term "manganese superoxide dismutase." The definition is provided at page 14, lines 35-38. Evidence to support the enablement of MNSOD genes other than SOD2 amplified by the primers of SEQ ID NOs: 1 and 2 has not been provided.

At pages 20-21, the response notes that compliance with the enablement requirement does not turn on the disclosure of a working example. Examples are not requires so long as the invention is otherwise disclosed in such a manner that one skilled in the art would have been able to practice the invention without an undue amount of experimentation. The response asserts that for the reasons discussed in the replies filed 7/1/2009 and 4/13/2009, undue experimentation is not required, and the claims satisfy the enablement requirement.

These arguments are not found persuasive for the reasons set forth above. Furthermore, the amount of guidance or direction needed to enable the invention is inversely related to the amount of knowledge in the state of the art as well as the predictability in the art. *In re Fisher*, 427 F.2d 833, 839, 166 USPQ 18, 24 (CCPA 1970). The "predictability or lack thereof" in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. If one skilled in the art can readily anticipate the effect of a change within the subject matter to which the claimed invention pertains, then there is predictability in the art. On the other hand, if one skilled in the art cannot readily anticipate the effect of a change within the subject matter to which that claimed invention pertains, then there is lack of predictability in the art. In the instant case, the evidence on the record indicates that the area of the invention was

unpredictable and one could not have extrapolated the results disclosed in the working example to other gene expression comparisons with the expectation of achieving a similar sensitivity and specificity for the detection of circulating cancer cells. Furthermore, the evidence disclosed in the working examples is not sufficient to enable the diagnosis of a tumor, recurrence, or metastases. The claimed invention is dependent upon the ability to reliably classify a single subject as having or not having disseminated cancer cells based upon gene expression comparisons, where the evidence on the record indicates that gene expression comparisons will give variable results dependent upon the genes used, detection method used, comparative sample used, etc. It is not obvious from the disclosure what other embodiments will work.

For these reasons, and the reasons made of record in the previous office actions, the rejection is maintained.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Christopher Low can be reached at 571-272-0951. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Jennifer Dunston/
Examiner
Art Unit 1636